DEPARTMENT OF HEALTH AND HUMAN SERVICES

What's New in the 2004 NCCLS Standards for Antimicrobial Susceptibility Testing?

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At the conclusion of this talk, you will be able to……

- List the major changes in the 2004 NCCLS tables (M100-S14)
- Describe new test/report recommendations for *Staphylococcus* spp. including:
  - testing for inducible clindamycin resistance
  - use of cefoxitin disk test to detect oxacillin-resistant staphylococci

At the conclusion of this talk, you will be able to……(con't)

- Discuss disk diffusion testing of *Stenotrophomonas maltophilia* and *Burkholderia cepacia*
- Describe the new reference guide for QC testing frequency when various test components are modified

NCCLS Standards - 2004

- **M100-S14 Tables (2004)** New!

.....to be used with text documents explaining how to perform the tests....

M2-A8 Disk Diffusion (2003)

M7-A6 MIC (2003)

NCCLS

Reference Terminology

….when I refer to….

- **M100 -- this means the new tables (M100-S14)**
- **M2 -- this means the disk diffusion method (described in M2-A8)**
- **M7 -- this means the MIC method (described in M7-A6)**

Major Changes 2004

NCCLS M100-S14

NCCLS

Updated information in M100-S14

Updated Information in This Edition

This document includes all of the tables from the NCCLS Disk Diffusion (M2) susceptibility testing document. There are several important changes to the tables that have resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing during 2003. Included below is a summary of the changes in this document, which supersede the tables published in 2002 and in earlier years.

Summary of Major Changes in This Document

The list includes the "major" changes in this document. Other minor or editorial changes have been made to the general formatting and to some of the table footnotes.

Additions/Changes

Introduction to Tables:

Organism *Yersinia pestis* - Added along with antimicrobial agents that must not be reported as susceptible (M7; Warning Table)

Suggested Grouping of Antimicrobial Agents:

Antimicrobial agents that should be considered for testing and reporting on potential agents of bioterrorism added (M7; Table 1B)

Enterobacteriaceae:

Major Changes

- ***Enterobacteriaceae***
  - More on *Salmonella* spp. and using nalidixic acid to screen for fluoroquinolone resistance
- ***Pseudomonas aeruginosa* and other non-Enterobacteriaceae**
  - Move levofloxacin from Test / Report Group “U” to Group “B”
  - Disk diffusion breakpoints for *Stenotrophomonas maltophilia* and *Burkholderia cepacia*

Major Changes (con't)

- ***Staphylococcus* spp.**
  - Inducible clindamycin resistance testing/reporting
  - Cefoxitin disk test for *mec*A
- **Coagulase-negative staphylococci**
  - More on *mec*A and oxacillin MIC results
  - More on reporting β-lactams on oxacillin susceptible isolates
- ***Enterococcus faecalis***
  - Predicting imipenem susceptibility from ampicillin results

Major Changes (con't)

- **Quality Control**
  - **Reference Guide** for QC testing frequency
  - QC ranges for *E. coli* ATCC 35218 and β-lactam / β-lactamase inhibitor combination agents when using Haemophilus Test Medium (HTM)
  - **Oritavancin** QC ranges
    - *Staphylococcus aureus* ATCC 29213
    - *Enterococcus faecalis* ATCC 29212
    - *Streptococcus pneumoniae* ATCC 49619

Major Changes (con't)

- **New breakpoints**
  - **Gemifloxacin**
    - ***Enterobacteriaceae*** **(FDA-approved for** ***Klebsiella pneumoniae)***
    - ***Haemophilus*** **spp.**
    - ***Streptococcus pneumoniae***
  - **Telithromycin**
    - ***Staphylococcus spp.***
    - ***Haemophilus*** **spp.**
    - ***Streptococcus pneumoniae***

Major Changes (con't)

- **Additions to Table 1A**
  - ***Haemophilus* spp.**
    - **Gemifloxacin (Group C)**
  - ***Streptococcus pneumoniae***
    - **Gemifloxacin (Group (B)**

Suggested Agents For Routine Testing and Reporting (Fastidious Organisms)

Table 1A. Suggested Groupings of U.S. FDA-Approved Antimicrobial Agents That Should Be Considered for Routine Testing and Reporting on Fastidious Organisms by Clinical Microbiology Laboratories

	Haemophilus spp.[a]	*Neisseria gonorrhoeae*	*Streptococcus pneumoniae*[j]	*Streptococcus* spp. Other Than *Streptococcus pneumoniae*
GROUP A PRIMARY TEST AND REPORT	Ampicillin[e,g]		Erythromycin[g]	Erythromycin[g,o]
	Trimethoprim-sulfamethoxazole		Penicillin (oxacillin disk)[k]	Penicillin[m,n,p] or ampicillin[n,p]
			Trimethoprim-sulfamethoxazole	
GROUP B[b] PRIMARY TEST REPORT SELECTIVELY	Cefotaxime[e] or ceftazidime[e] or ceftizoxime[e] or ceftriaxone[e]		Clindamycin	Chloramphenicol[o]
			Gatifloxacin or levofloxacin or moxifloxacin or sparfloxacin **Gemifloxacin** Ofloxacin	
	Cefuroxime sodium (parenteral)		Tetracycline[d]	
	Chloramphenicol[e]			

Table 1A

Major Changes (con't)

- **Potential agents of bioterrorism**
  - **Separate tables for this group of organisms**
  - ***Bacillus anthracis, Yersinia pestis, Burkholderia mallei, Burkholderia pseudomallei***

Major Changes M100-S14

GNR

GNR

Salmonella and Fluoroquinolones (FQ)

- "FQ-susceptible strains of Salmonella that test resistant to nalidixic acid may be associated with clinical failure or delayed response in FQ-treated patients with extraintestinal salmonellosis. Extraintestinal isolates of Salmonella should also be tested for resistance to nalidixic acid. For isolates that test susceptible to FQs and resistant to nalidixic acid, the physician should be informed that the isolate may not be eradicated by FQ treatment. A consultation with an infectious disease practitioner is recommended."

M100-S14 (M2, M7); Table 2A

Salmonella spp. (blood)

	MIC (μg/ml)
ampicillin	>32 R
ciprofloxacin	≤0.25 S
ceftriaxone	≤0.5 S
trimeth-sulfa	>4/78 R

….Test nalidixic acid on extraintestinal isolates with ciprofloxacin MICs of 0.12–1.0 μg/ml; a ciprofloxacin MIC of 2.0 μg/ml is Intermediate and ≥4.0 μg/ml is Resistant

Salmonella and Ciprofloxacin

CIP MIC (μg/ml)	NCCLS Interpretation	Likely Mutation	Nalidixic acid
≤ 0.06	S	none	S
0.12- 1	S	one	R*
≥ 4	R	two	R

***Some patients with extraintestinal infection with *Salmonella* spp. may fail FQ therapy; use nalidixic acid as a surrogate to detect single step mutants.**

Threlfall et al. 2001. EID. 7:448.
Butt et al. 2003. EID 9:1621.

Salmonella spp. (blood)

	MIC (μg/ml)
ampicillin	>32 R
ciprofloxacin	≤0.25 S*
ceftriaxone	≤0.5 S
trimeth-sulfa	>4/78 R

….if nalidixic acid is resistant, add comment such as……

”This isolate demonstrates reduced susceptibility to fluoroquinolones. For some patients with extraintestinal Salmonella infections with such isolates, the isolates may not be eradicated by fluoroquinolone treatment. ID consult suggested.”

Salmonella and Ciprofloxacin

- **"*Salmonella* spp. isolated from sterile sites or from patients that have failed FQ therapy should be tested for the MIC of ciprofloxacin or susceptibility to nalidixic acid. Those isolates for which the ciprofloxacin MICs are ≥0.125 μg/ml or resistant to nalidixic acid should be considered to have reduced susceptibility to FQs and physicians should be warned that clinical failure or delayed response may be associated with FQ treatment of infections caused by these isolates".**

Poutanen and Low. 2003. CMN 25:97

Salmonella and Reduced Ciprofloxacin Susceptibility (MIC ≥0.12 μg/ml)

- Isolates uncommon in USA
  (www.cdc.gov/narms/annual/2001)
- UK study (1999) - 23% in *S. typhi*
  - Mostly travelers from India and Pakistan

  Threlfall et al. 2001. EID. 7:448.
- Nalidixic acid screen study - n=1010 Salmonella; 50 isolates w/ reduced ciprofloxacin susceptibility:
  - Sensitivity 100%
  - Specificity 87%

  Hakanen et. al. 1999. JCM. 37:3572.

New Disk Diffusion Breakpoints

- ***Stenotrophomonas maltophilia***
  - levofloxacin
  - minocycline
  - trimethoprim-sulfamethoxazole
- ***Burkholderia cepacia***
  - ceftazidime
  - meropenem
  - minocycline

35°C; ambient air; 20-24 h incubation

Excerpt from Table 2B (M2)….

"Zone Diameter Interpretive Standards and Equivalent MIC Breakpoints for *P. aeruginosa*, *Acinetobacter* spp., *S. maltophilia*, and *B. cepacia*"

Agent	R	I	S	Comments
Ceftazidime	≤ 14 ≤ 17	15-17 18-20	≥ 18 ≥ 21	For *B. cepacia*
Minocycline	≤ 14	15-18	≥ 19	May be reported for *S. maltophilia* and *B. cepacia* also
Trimeth-sulfa	≤ 10	11-15	≥ 16	May be reported for *S. maltophilia* also

Relocation of Levofloxacin in Table 1 for *Pseudomonas aeruginosa* and Other Non-Enterobacteriaceae

…new M100-S14

Group B **Primary Test** **Report Selectively**	**ciprofloxacin** **levofloxacin**

…old M100-S13

Group U **Supplemental for Urine Only**	**levofloxacin or lomefloxacin or norfloxacin or ofloxacin**

M100-S14 (M2, M7); Table 1 & 2B

Major Changes M100-S14

Staphylococcus

Staphylococcus

Staphylococcus spp.

- "Macrolide resistant isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. may have constitutive or inducible resistance to clindamycin [methylation of the 23S rRNA encoded by the *erm* gene also referred to as MLS_B (macrolide, lincosamide, and type B streptogramin) resistance] or may be resistant only to macrolides (efflux-mechanism encoded by the *msrA* gene)."

M100-S14 (M2, M7); Table 2C

Staphylococcus spp.
Erythromycin / Clindamycin

Mechanism	Determinant	Erythro	Clinda
Efflux	*msrA*	R	S
Ribosome alteration	*erm*	R	S*
Ribosome alteration	*erm*	R	R constitutive

msrA = macrolide streptogramin resistance
erm = erythromycin ribosome methylase

*requires induction to show resistance

Staphylococcus aureus

clindamycin	S
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

If clindamycin-S and erythromycin-R, do not report clindamycin-S without performance of "D Test"

Optional reporting strategy

Staphylococcus aureus

erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

“Contact laboratory if clindamycin results needed”

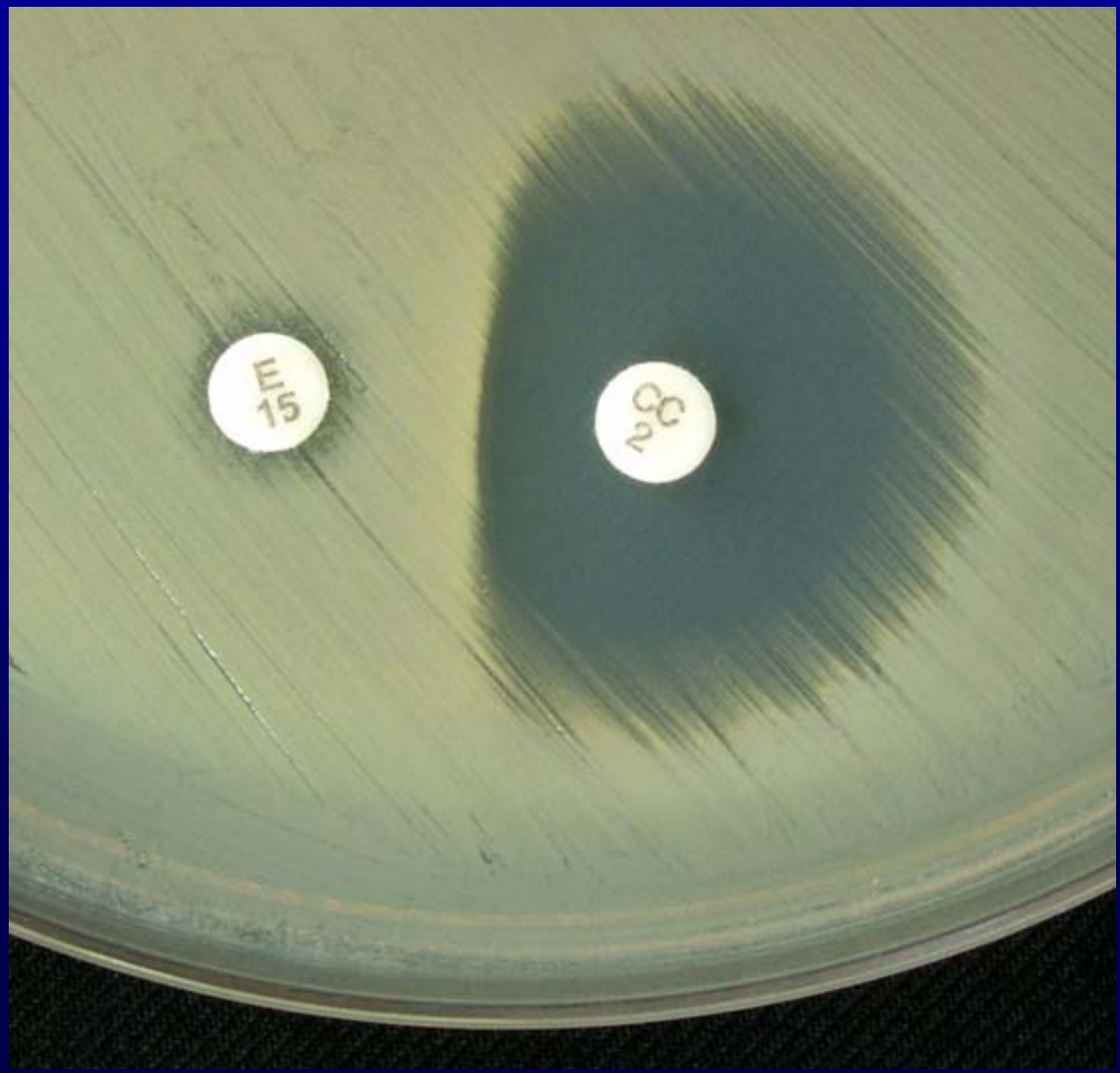
“D Test” – positive reaction

Inducible clindamycin resistance (*erm*-mediated)

Routine disk diffusion test:

Place 2 μg clindamycin disk 15 mm to 26 mm from edge of 15 μg erythromycin disk.

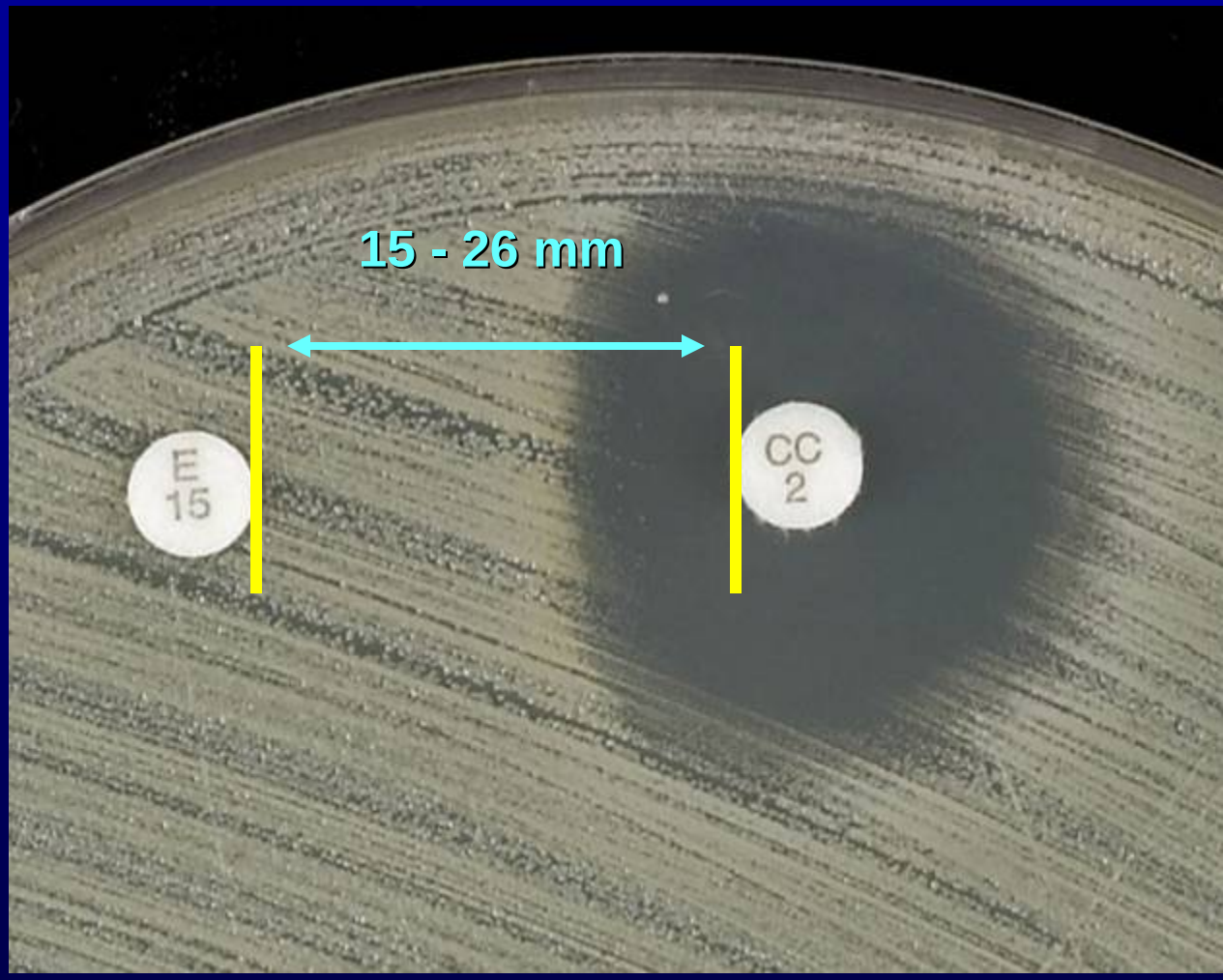
QC strain forthcoming!



“D Test” – positive reaction

Inducible clindamycin resistance (*erm*-mediated)

…another example

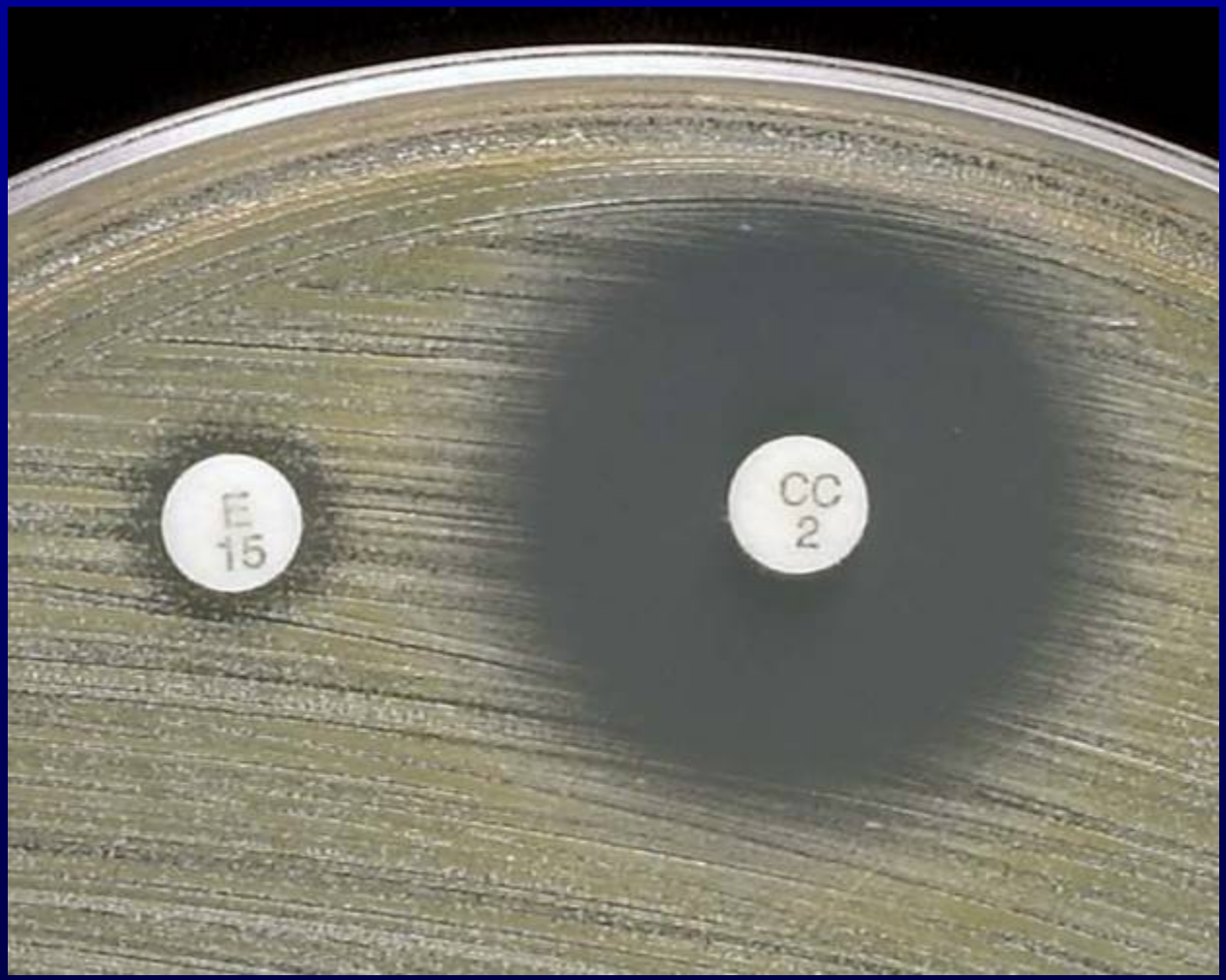


Photos courtesy of J. Jorgensen and K. Fiebelkorn.

“D Test” – negative reaction

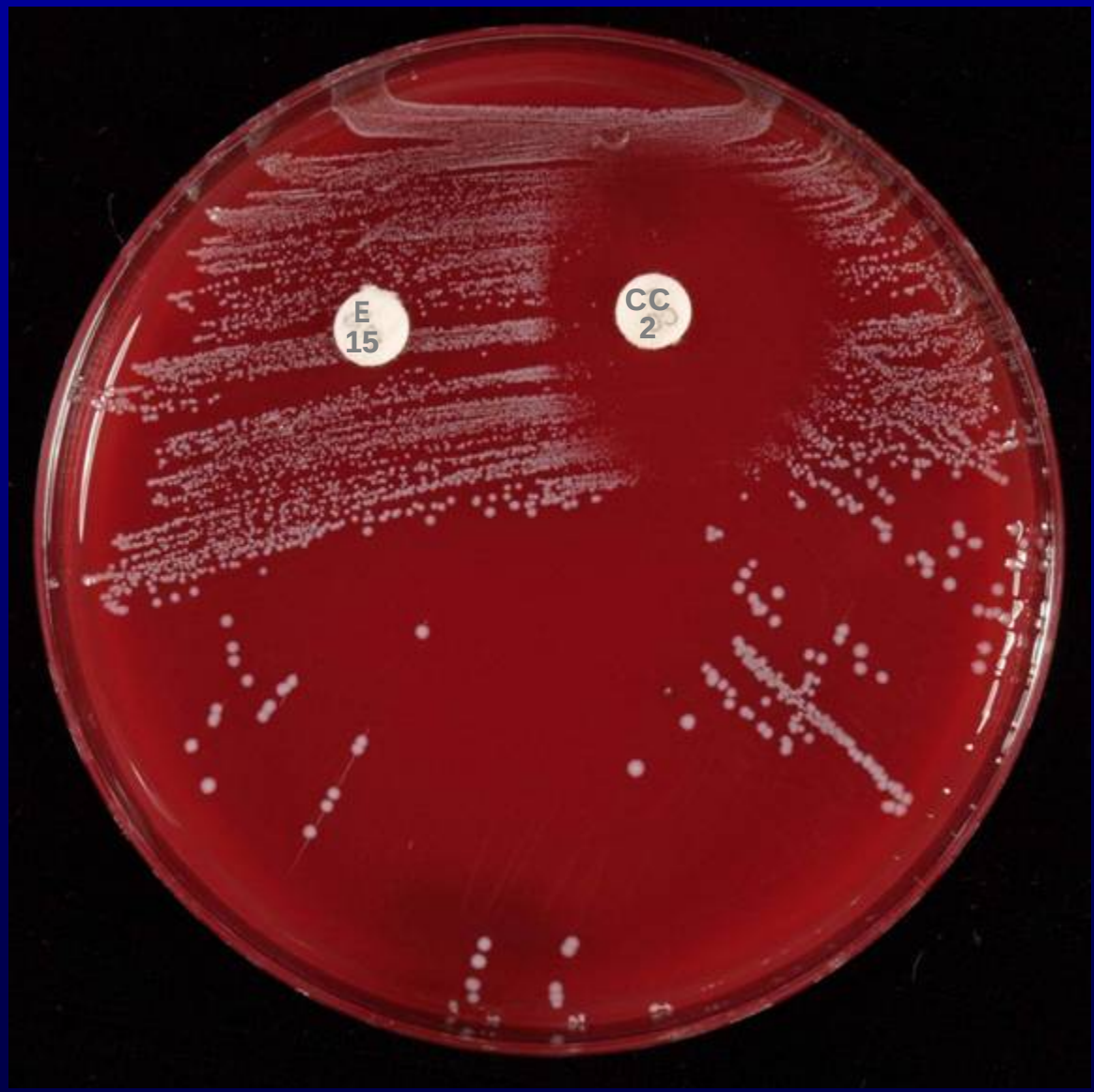
NO induction

(*msr*A-mediated erythromycin resistance)



“D Test” – positive reaction

Inducible clindamycin resistance (*erm*-mediated)



Routine purity plate:

- **Streak 1/3 of plate for confluent growth**
- **Place 2 μg clindamycin disk 15 mm from edge of 15 μg erythromycin disk**

"D Test" positive and optional comment

Staphylococcus aureus

clindamycin	R
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

"This *S. aureus* is presumed to be resistant based on detection of inducible clindamycin resistance. Clindamycin may still be effective in some patients."

"D Test" negative and optional comment

Staphylococcus aureus

clindamycin	S
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

"This *S. aureus* DOES NOT demonstrate inducible clindamycin resistance in vitro."

Inducible Clindamycin Resistance - Incidence

- **Varies considerably geographically**
- **Community-associated MRSA**
  - **Frequently erythromycin-R clindamycin-S**
  - **Often *msrA*-mediated mechanism (NOT inducible)**
- **USA report 2002**
  - **617 *S. aureus* erythromycin-R clindamycin-S**
  - **50% NOT inducible resistance**

    Fiebelkorn et al. 2003. JCM. 41:4740.

Staphylococcus spp.

- "The results of disk diffusion tests using a 30 μg cefoxitin disk and alternate breakpoints (see box at end of this table) can be used to predict *mec*A –mediated resistance in staphylococci."

M100-S14 (M2, M7); Table 2C

Disk Diffusion Screen for *mec*A-mediated Resistance in Staphylococci

- Perform standard disk diffusion test with cefoxitin (30 μg) disk
- Incubate 24 h; however, results may be reported after 18 h, if resistant
- Report results for **OXACILLIN**, not cefoxitin

Staphylococcus spp.

Table 2C. (Continued)

Disk Diffusion Screening Test[a] for Prediction of *mec*A-mediated Resistance in Staphylococci

Antimicrobial Agent (Disk Content)	Organism Group	Zone Diameter, Nearest Whole mm		Comments
Cefoxitin (30 µg)	*S. aureus*	≤ 19	≥ 20	(29) *S. aureus* for which cefoxitin disk diffusion zones are ≤ 19 mm should be reported as oxacillin resistant. Those for which cefoxitin zones are ≥ 20 mm should be reported as oxacillin susceptible.
	Coagulase-negative staphylococci	≤ 24	≥ 25	(30) Coagulase-negative staphylococci for which cefoxitin disk diffusion zones are ≤ 24 mm should be reported as oxacillin resistant. Those for which cefoxitin zones are ≥ 25 mm should be reported as oxacillin susceptible.

[a] Use standard disk diffusion testing conditions and incubate for 24 hours; however, results may be reported after 18 hours incubation if resistant.

Table 2C (M2, M7)

Disk Diffusion Screen for *mecA*-mediated Resistance in Staphylococci (con't)

	Cefoxitin zone (mm)	
S. aureus	≤ 19*	≥ 20**
CoNS	≤ 24*	≥ 25**

* Report as oxacillin resistant

** Report as oxacillin susceptible

CoNS, coagulase-negative staphylococci

M100-S14 (M2, M7); Table 2C

Staphylococcus - Oxacillin

MIC (µg/ml):

	Susc	Int	Res
S. aureus	≤ 2	-	≥ 4
CoNS	≤ 0.25	-	≥ 0.5

DD (mm):

	Res	Int	Susc
S. aureus	≤ 10	11-12	≥ 13
CoNS	≤ 17	-	≥ 18

M100-S14 (M2, M7); Table 2C

Oxacillin Breakpoints
Coagulase-Negative Staphylococci

- May **overcall resistance** for species other than *S. epidermidis* (e.g. *S. lugdunensis, S. saprophyticus*)
- For **serious infections** with CoNS other than *S. epidermidis*, testing for *mecA* or PBP 2a may be appropriate for strains having oxacillin MICs of 0.5 – 2 μg/ml or oxacillin zones ≤17 mm
- If *mec*A or PBP 2a negative, report as oxacillin susceptible

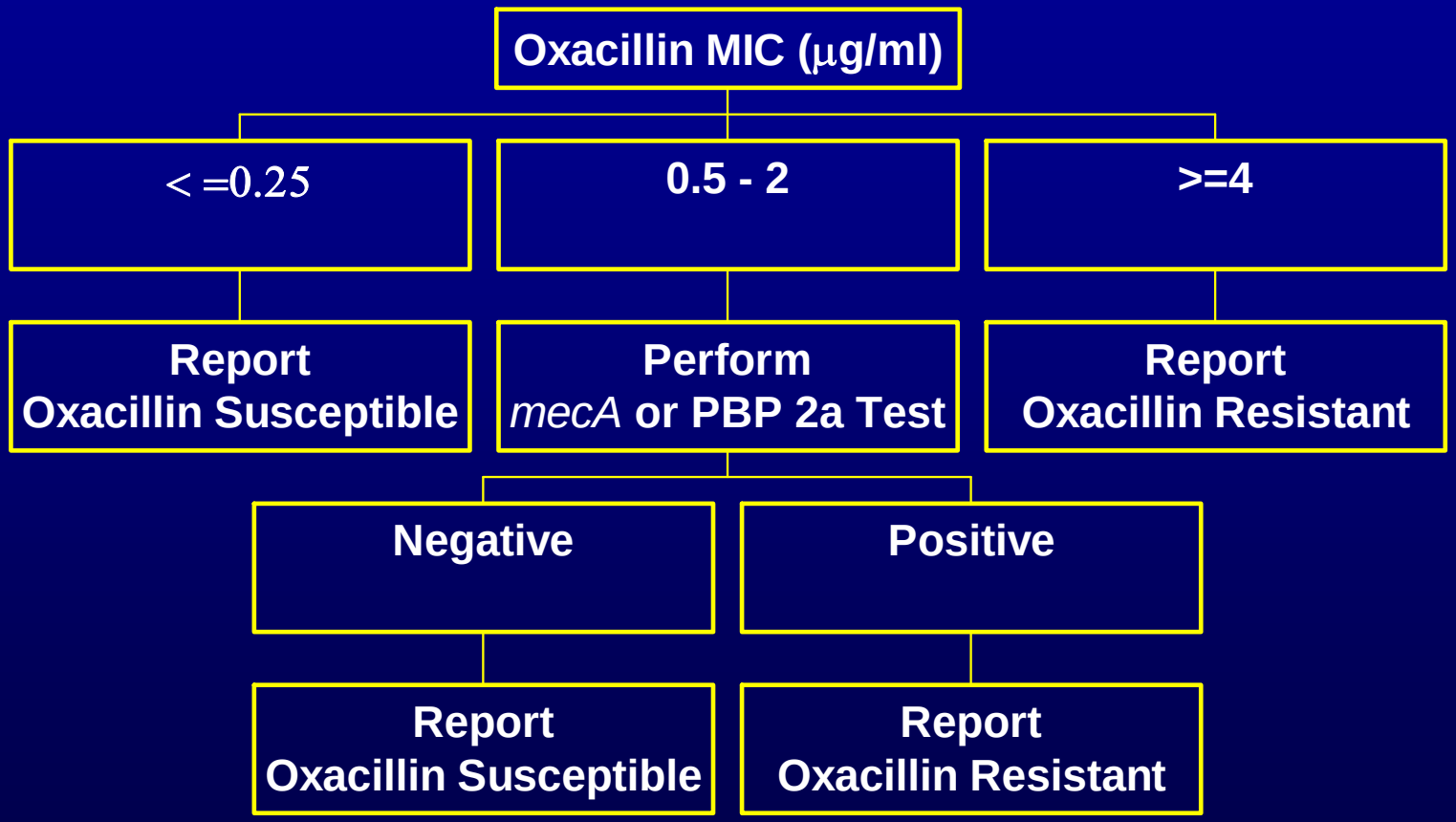
M100-S14 (M2, M7); Table 2C

Oxacillin Breakpoints
Coagulase-Negative Staphylococci (con't)

- For **oxacillin-resistant** strains (including PBP 2a or *mec*A positive strains), report all β-lactams resistant
- For **oxacillin-susceptible** strains, report any β-lactams tested according to results generated

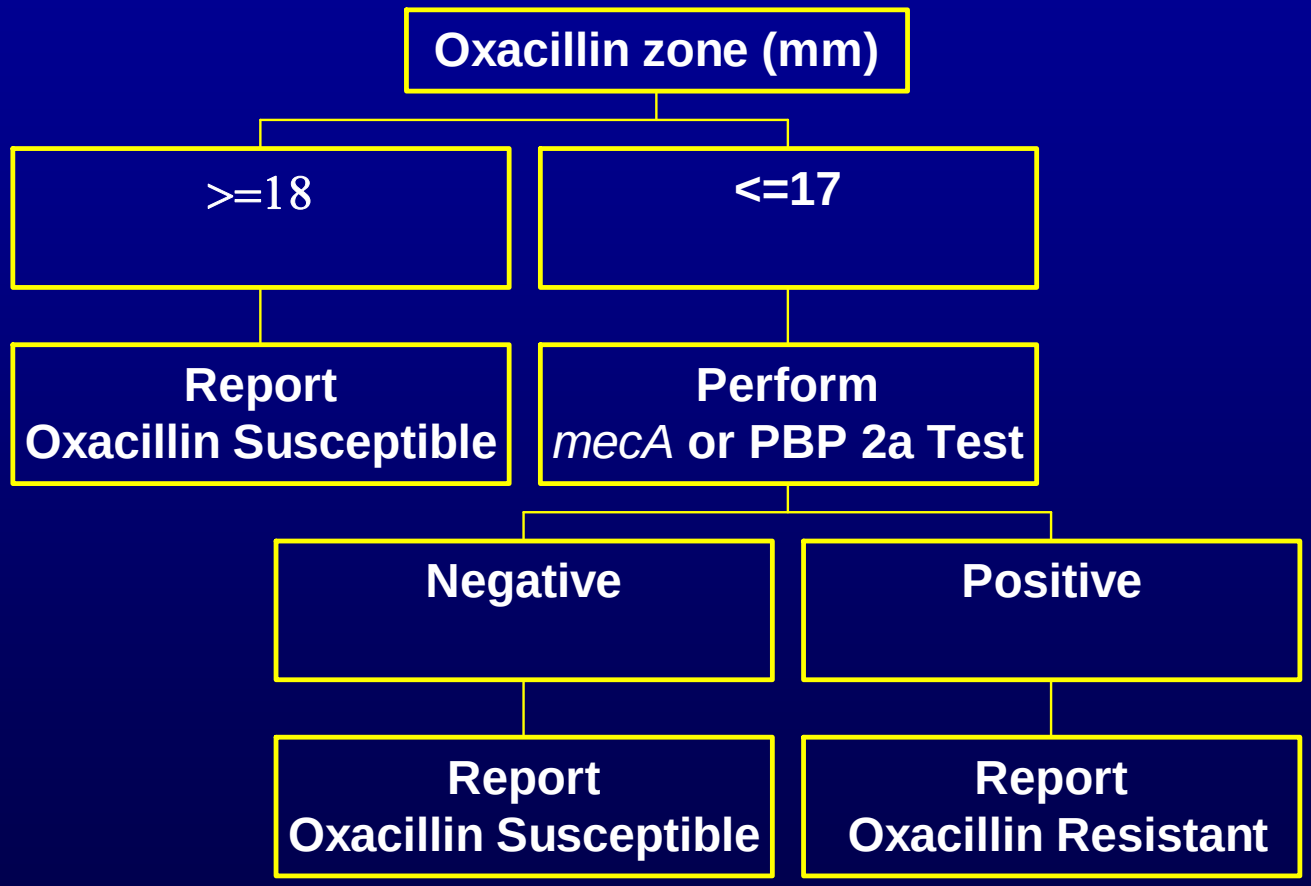
M100-S14 (M2, M7); Table 2C

Reporting Oxacillin MIC Results for Coagulase-Negative Staphylococci*



*For testing non-*S. epidermidis* from sterile sites

Reporting Oxacillin Disk Diffusion Results for Coagulase-Negative Staphylococci*



*For testing non-*S. epidermidis* from sterile sites

Major Changes M100-S14

Enterococcus faecalis

E. faecalis

Enterococcus faecalis

- **"Ampicillin susceptibility can be used to predict imipenem susceptibility provided the species is confirmed to be *E. faecalis*".**

M100-S14 (M2, M7); Table 2D

If MD requests imipenem results on E. faecalis….

E. faecalis (blood)

ampicillin	S
vancomycin	S
gent synergy	R
strep synergy	S

"Ampicillin-susceptible *E. faecalis* are imipenem susceptible"

Major Changes M100-S14 Quality Control

QC

Reference Guide to Quality Control Testing Frequency

Table 3B (M2, M7)

Table 3B. Reference Guide to Quality Control Testing Frequency

This table summarizes the suggested frequency of testing NCCLS-recommended ATCC quality control strains to be performed by the user of antimicrobial susceptibility tests (AST). It applies only to antimicrobial agents for which 20 or 30 consecutive test days of quality control testing produced satisfactory results.

Test Modification	Number of days of consecutive QC testing required			Comments
	1	5	20 or 30	
MIC Test(s)				
Use new shipment or lot number	X			
Expand dilution range	X			Example: Convert from breakpoint to expanded range MIC panels.
Reduce dilution range	X			Example: Convert from expanded dilution range to breakpoint panels.
Use new method (same company)			X	Examples: Convert from visual to instrument reading of panel. Convert from overnight to rapid MIC test. In addition, perform in-house validation studies.
Use new manufacturer of MIC test			X	In addition, perform in-house validation studies.
Inoculum Preparation				
Convert inoculum preparation/ standardization to use of a device that has its own QC protocol		X		Example: Convert from visual adjustment of turbidity to use of a photometric device for which a quality control procedure is provided.
Convert inoculum preparation/ standardization to a method that is dependent on user technique			X	Example: Convert from visual adjustment of turbidity to another method that is not based on a photometric device.
Instrument/Software				
Software update that affects AST results		X		Monitor all drugs, not just those implicated in software modification.
Repair of instrument that affects AST results	X			Depending on extent of repair (e.g., critical component such as the optics) additional testing may be appropriate (e.g., 5 days).

NOTE 1: Addition of any NEW antimicrobial agent requires 20 or 30 consecutive days of satisfactory testing (see M7-A6 Section 12.7) prior to use of this guide.

NOTE 2: QC can be performed prior to or concurrent with testing patient isolates. Patient results can be reported for that day if quality control results are within the acceptable limits.

NOTE 3: Manufacturers of commercial or in-house prepared tests should follow their own internal procedures and applicable regulations.

NOTE 4: For troubleshooting out-of-range results, refer to M7-A6, Section 12.9.

NOTE 5: Broth, saline, and/or water used to prepare an inoculum does not require routine quality control.

Excerpt from: "Reference Guide to QC Testing Frequency"

(for ATCC QC strains after 20-30 consecutive days of satisfactory daily testing)

	No. of days of consecutive QC testing required			
MIC test modification	1	5	20 or 30	Comments
Use new shipment or lot number	X			
Use new manufacturer of MIC test			X	In addition, perform in-house validation studies
Software update that affects AST results		X		Monitor all drugs not just those implicated in software modification

M100-S14 (M2, M7); Table 3B

Excerpt from: "Reference Guide to QC Testing Frequency" (con't)

- **Note 1:** Addition of any NEW antimicrobial agent requires 20 or 30 consecutive days of satisfactory testing, prior to use of this guide.
- **Note 2:** QC can be performed prior to or concurrent with testing patient isolates. Patient results can be reported for that day if QC results are within the acceptable limits.

M100-S14 (M2, M7); Table 3B

Excerpt from: "Reference Guide to QC Testing Frequency" (con't)

- **Note 3:** Manufacturers of commercial or in-house prepared tests should follow their own internal procedures and applicable regulations.
- **Note 4:** For troubleshooting out-of-range results, refer to M2-A8 or M7-A6, QC section.
- **Note 5:** Broth, saline and/or water used to prepare an inoculum does not require routine QC.

M100-S14 (M2, M7); Table 3B

More Examples…..
Application of Table 3C
(consecutive days of daily QC required)

- **Inoculum preparation, convert from:**
  - Visual to Prompt – 20 or 30 days
  - Visual to photometer – 5 days
  - Prompt to photometer – 5 days
- **Instrument / software**
  - Repair of instrument – 1 day (or more)
  - Replace hardware (e.g. reader-incubator) – 20 or 30 days

Haemophilus Test Medium (HTM)

- Addition of disk diffusion and MIC QC ranges for *E. coli* ATCC 35218 (ß-lactamase producing strain) and ß-lactam / ß-lactamase inhibitor drugs

QC Ranges Using HTM

	H. influenzae ATCC 49247	E. coli ATCC 35218
Zone (mm)		
Amoxicillin-clavulanic acid	15 - 23 mm	17 - 22 mm
MIC (μg/ml)		
Amoxicillin	-	≥ 256
Amoxicillin-clavulanic acid	2/1 – 16/8	4/2 - 16/8
Ticarcillin-clavulanic acid	-	16/2 – 64/2

M100-S14 (M2, M7); Table 3A

Major Changes M100-S14

Newer Antimicrobial Agents

New Agents

Newer Antimicrobial Agents…

Agent	Drug class	Route of administration	FDA approved
Daptomycin (Cubicin)	lipopeptide	IV	yes
Gemifloxacin (Factive)	fluoroquinolone	PO	yes
Oritavancin	glycopeptide	IV	no
Telithromycin (Ketek)	ketolide	PO	no

See Glossary in M100-S14

Daptomycin

- In vitro activity against gram-positive bacteria including MRSA and VRE
- Mode of action
  - Bactericidal
  - Requires physiologic calcium

Daptomycin (con't)

- Susceptibility testing media requirements
  - Mueller-Hinton broth – 50 mg/L calcium chloride
  - Mueller-Hinton agar – 28 mg/L calcium chloride
- Currently, no NCCLS breakpoints; FDA breakpoints available in product literature
- NCCLS QC ranges available
- No resistance reported to date among *S. aureus* or Group A or B Streptococcus

Gemifloxacin

- Active against respiratory pathogens including *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila*
- Inhibits DNA synthesis through inhibition of both DNA gyrase and topoisomerase IV (dual target)

Oritavancin

- Bactericidal in vitro
- Active against most gram-positive pathogens including VRE

Telithromycin

- Active against respiratory pathogens (*S. pneumoniae, H. influenzae, M. catarrhalis, Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila)*
- Active against other gram-positive bacteria that have inducible MLS_B mechanism of resistance and does not induce resistance
- Active against *S. pneumoniae* resistant to erythromycin and clarithromycin regardless of resistance mechanism

Major Changes M100-S14 Bioterrorism Agents

Bioterrorism

Bioterrorism Agents

Table 2K. MIC Interpretive Standards (µg/mL) for Potential Agents of Bioterrorism: *Bacillus anthracis, Yersinia pestis, Burkholderia mallei,* and *Burkholderia pseudomallei*

Testing Conditions	
Medium:	Broth dilution: Cation-adjusted Mueller-Hinton broth (CAMHB)
Inoculum:	**Growth method or direct colony suspension in CAMHB,** equivalent to a 0.5 McFarland standard
Incubation:	35 °C; ambient air; 16 to 20 hours; **for *Yersinia pestis* incubate 24 hours and if unacceptable growth in the control well reincubate an additional 24 hours.**

Minimal QC Recommendations (See Table 3 for acceptable QC ranges.)

Escherichia coli ATCC® 25922 **(all organisms)**
***Escherichia coli* ATCC® 35218 (for amoxicillin-clavulanic acid and *Burkholderia pseudomallei*)**
Staphylococcus aureus ATCC® 29213 **(for *Bacillus anthracis* only)**
***Pseudomonas aeruginosa* ATCC® 27853 (for *Burkholderia mallei/ pseudomallei* only)**

General Comments

(1) **Extreme Caution:** Public health officials should be notified about all isolates presumptively identified as *B. anthracis, Y. pestis, B. mallei, or B. pseudomallei.* Confirmation of isolates of these bacteria may require specialized testing only available in reference or public health laboratories. Recommended precautions: Biosafety Level 2 (BSL2) practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. Biosafety Level 3 (BSL3) practices, containment equipment, and facilities are recommended for work involving production quantities or concentrations of cultures, and for activities with a high potential for aerosol production. If BSL2 or BSL3 facilities are not available, isolates should be forwarded to reference or public health laboratories for susceptibility testing.

(2) Interpretive criteria are proposed based on population distributions, pharmacokinetics of the antimicrobial agents, previously published literature, and animal model data.

(3) Criteria for *B. anthracis* do not apply to other *Bacillus* spp.

(4) WARNING: For *Yersinia pestis,* studies have demonstrated that although β-lactam antimicrobial agents may appear active *in vitro* they lack efficacy in animal models of infection. These antimicrobial agents should not be reported as susceptible.

NOTE: Information in boldface type is considered tentative for one year.

Organism Group	Antimicrobial Agent	MIC (µg/mL) Interpretive Standard S	I	R	Comments
PENICILLINS					
	Penicillin	≤ 0.12	-	≥ 0.25	(5) Class representative for amoxicillin.
CTAMASE INHIBITOR COMBINATIONS					
	Amoxicillin-clavulanic acid	**≤ 8/4**	**16/8**	**≥ 32/16**	

Table 2K (M7)

Potential Agents of Bioterrorism

- **Bacteria included in M100-S14**
  - ***Bacillus anthracis***
  - ***Burkholderia mallei***
  - ***Burkholderia pseudomallei***
  - ***Yersinia pestis***
- **New Tables**
  - **1B (Antimicrobial agents to test/report)**
  - **2K (MIC interpretive standards)**

Summary of Comments and Subcommittee Responses

NCCLS consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

M100-S13: *Performance Standards for Antimicrobial Susceptibility Testing; Thirteenth Informational Supplement* (M7-MIC Testing)

Table 2A

1. For *Klebsiella* spp. and *E. coli*, the NCCLS most recent susceptibility performance standards (M100) instruct laboratories to report "cephalosporins" as resistant regardless of the MIC value or zone size if ESBL production is confirmed to be positive for these strains. Footnote c of the Glossary I table suggests that cefepime is to be included as a member of the "cephalosporin" group and by deduction should be reported as resistant on confirmed positive ESBL-producing isolates. Is this the M100 standard's intended interpretation?

- **Yes. Available data indicate that cefepime can be hydrolyzed by some ESBLs. Although there are some reports of efficacy of this agent against these strains, available data overall indicate that strains with confirmed ESBL production should be reported as resistant to cefepime.**

2. Clinical observations by some of our Infectious Diseases physicians in our facility raise concerns over the clinical efficacy of treating ESBL-producing strains of *Klebsiella pneumoniae* and *E. coli* with cephamycins. Clinical failures have been reported both in-house and in the literature, and the appropriateness of reporting these agents as "susceptible" (even when ESBL production has been confirmed) has been challenged. On what basis does NCCLS uphold the efficacy of employing cephamycins for the treatment of ESBL-producing strains? Is there any current trend within NCCLS to change or at least question the status of cephamycins in this context?

Very last pages of M100-S14!

Recap of ..“Summary of Comments and Subcommittee Responses”

- **ESBLs testing**
  - Report cefepime as resistant for ESBL producers
  - There is limited data on use of cephamycins (e.g. cefoxitin, cefotetan) for treating infections caused by ESBL producers
  - If ESBL confirmatory test negative, report results as tested (do not override to resistant)
  - Currently, only *E. coli* and *Klebsiella* spp. are addressed in NCCLS ESBL testing rules

Recap of..“Summary of Comments and Subcommittee Responses” (con’t)

- **Other GNR**
  - There are no specific NCCLS recommendations for testing for inducible β-lactamases. To help detect resistance to 3rd generation cephalosporins resulting from selection of derepressed mutants, repeat testing after 3-4 days is suggested for *Enterobacter*, *Citrobacter*, *Serratia*.
- ***mecA*** **and coagulase-negative staphylococci**
  - Discussed above

Recap of..“Summary of Comments and Subcommittee Responses” (con’t)

- **Incubation temperature**
  - range 33-35°C
  - oxacillin – *Staphylococcus* spp., 33-35°C (not >35°C)
- ***Haemophilus* spp.**
  - β-lactamase testing only would not detect BLNAR strains
- **MIC testing –frequency of performing colony count to QC inoculum**
  - Perform at least quarterly
- **QC of commercial McFarland standards**
  - Follow manufacturers recommendations

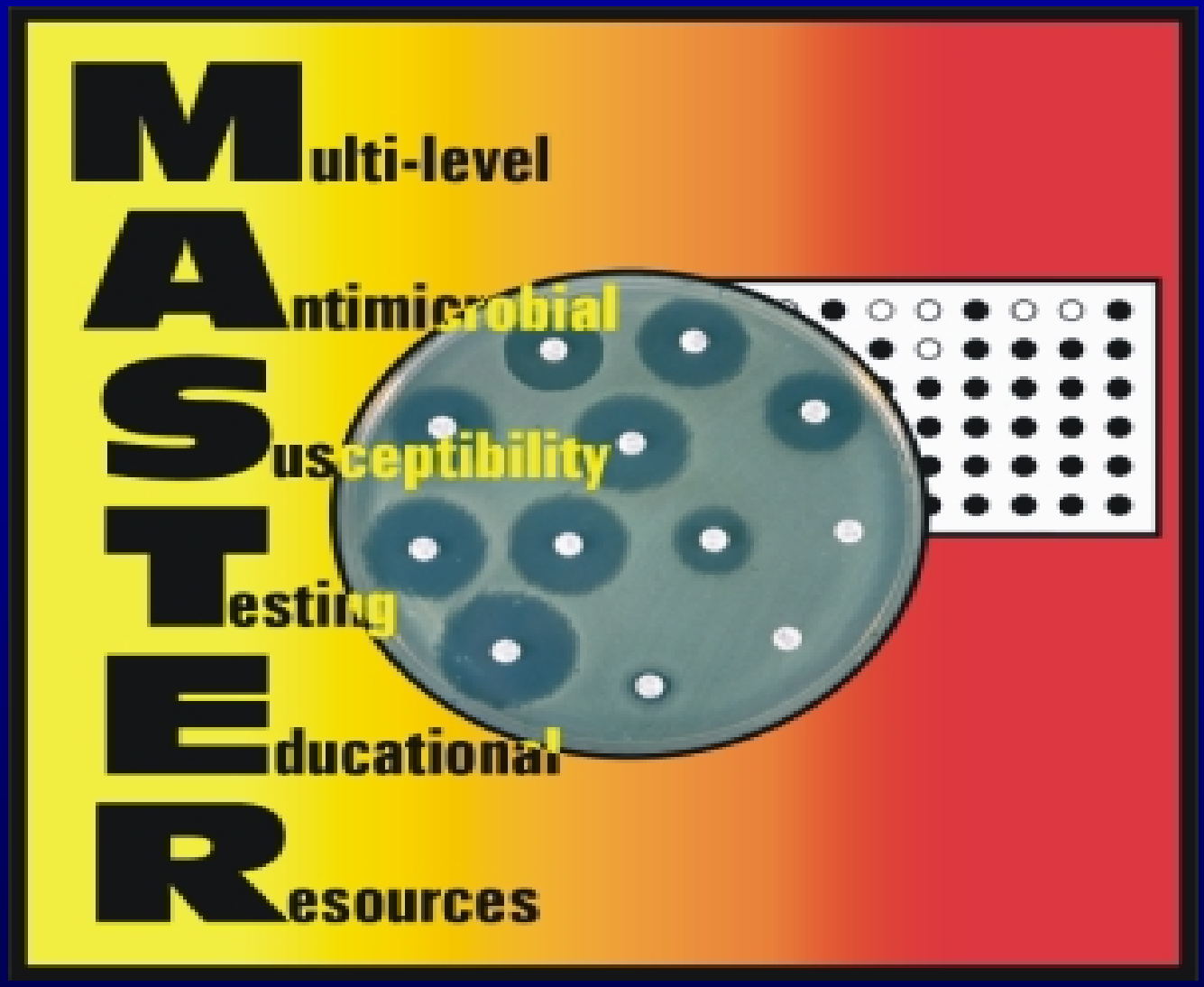
Some Issues Under Discussion by NCCLS

- *Staphylococcus* spp. - re-evaluate moxifloxacin, gatifloxacin, levofloxacin, ciprofloxacin breakpoints
- Acinetobacter – examine correlation of disk and MIC results for β-lactams and tetracyclines
- Development of new Guideline for testing bacteria not currently addressed in NCCLS AST standards (e.g. *Corynebacterium*, HACEK, etc.)

Enterobacteriaceae β-Lactam Breakpoints and ESBL Issues

- Re-evaluation of β-lactam breakpoints for Enterobacteriaceae
  - Example: cefotaxime
    - Current – Susceptible at ≤ 8 μg/ml
    - Proposed – Susceptible at ≤ 1 or ≤ 2 μg/ml
  - Substantial data needed
  - Goal is to more accurately detect all β-lactamase and other β-lactam resistance mechanisms with revised breakpoints
- Changing breakpoints – commercial systems project it will take 3 years …much $$$$$!

Thank you!



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